의학석사 학위논문

# 위의 초고분화 선암의 진단적병리소견의 연구

# Extremely Well-Differentiated Adenocarcinoma of the Stomach: Diagnostic Pitfalls in Endoscopic Biopsy

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# Extremely Well-Differentiated Adenocarcinoma of the Stomach: Diagnostic Pitfalls in Endoscopic Biopsy

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2021년 8월

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# 국 문 요 약

위의 초고분화 선암 (Extremely Well-Differentiated Adenocarcinoma, EWDA)은 매우 분화가 좋은 암이며, 특히나 위의 내시경적 생검조직에서 진단시 어려움이 있으며 희소한 종양이어서 기존의 연구가 많지 않다. 이의 진단적 병리소견을 분석해보기위해 여러 케이스의 EWDA 를 모아 조사해보았다. 19명의 환자의 총 55개 조직을 리뷰하였으며 육안, 병리소견 모두 기록하여 분석하였다. 또한, 병리적으로 현미경 소견이 유사한 주변 정상 Foveolar epithelium, regenerative atypia, hyperplastic polyp, and foveolar epithelial hyperplasia specimens 들과 비교하였다.

연구 결과, 대부분의 경우는 진행성 위암 (18 of 19, 94.7%) 이었으며 위체부에 위치하였다 (15 of 19, 79.0%). 또한 대부분의 진행성 위암 케이스는 궤양을 동반하지 않았다 (12 of 18, 66.7%). 특징적으로 병변은 종괴를 형성하며 밑으로 침투하는 듯이 자라나서 점막을 실제 침범하는 부분은 상대적으로 적었다. 진단적 현미경 소견으로는 Irregular glandular shape, undulating apical cytoplasmic border, disproportionately large glands, irregularly spaced nuclei and variably distended mucinous cytoplasm 가 관찰되었고 일반적인 위선암에서 보이는 demoplastic reaction을 동반한 침윤선은 드물었다.

결론적으로, 위의 초고분화 선암은 정상점막의 소견과 매우 유사하게 생겨 진단이 종종 지연되기도 한다. 이런 독특한 종양의 특징을 이해하는 것이 향후 빠른 진단과 치료에 도움이 될 것으로 생각된다.

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# 제 1 장 서 론 (Introduction)

Extremely well-differentiated adenocarcinoma of the stomach is rare and is histologically almost indistinguishable from benign hypertrophic glands. It is both understudied and diagnostically challenging due to its bland nuclear features and subtle architectural atypia. Intestinal- and gastric-type EWDAs are its subgroups, mimicking intestinal metaplasia and normal foveolar epithelium, respectively. Intestinal-type EWDAs consist of intestinal-type glands with various amounts of goblet and Paneth cells[1]. Gastric-type EWDAs are described as mucin-rich columnar cells with basally located, bland-looking nuclei mimicking hyperplastic foveolar epithelium or dilated pyloric glands[2].

Due to its rather featureless morphology, misinterpretation of EWDA as benign in gastric forceps biopsy samples is fairly common[2], and failure to identify it correctly leads to delays in therapeutic procedures. There have been reports in the literature discussing its histomorphologic features, yet reported cases with a comprehensive histologic review are scarce. In our institution, we collected 19 cases of EWDA, extracted their key gross, microscopic features along with clinical data and follow-up information, and discussed its microscopic features. These findings will be helpful in the clinic, especially when diagnosing small preoperative forceps biopsy samples.

# 제 2 장 본 문 (Body)

## 2.1 Materials and Methods

### 2.1.1 Case selection

Fourteen EWDAs were acquired from the authors' institutional archive from 2018 to 2021. All cases met the previously discussed criteria by Yao et al.[1] and we adopted that definition with a slight modification. EWDA was defined as neoplastic glands comprised of highly differentiated cells mimicking intestinal metaplasia or normal gastric foveolar epithelium and mild nuclear atypia. All 19 cases were reviewed by two pathologists for confirmation.

To estimate prevalence of EWDA, we also reviewed 608 consecutively resected advanced gastric cancer (AGC)s treated at our institution in 2010. AGCs were selected because most of the archived cases were AGCs. Five of them (5 of 608, 0.08%) fulfilled the criteria for EWDA. Patient characteristics, surgical and endoscopic findings with follow-up data were obtained from the medical records of Asan Medical Hospital. Our study was approved by the Institutional Review Board of the Asan Medical Center (#2021-0485).

### 2.1.2 Endoscopic assessment

Endoscopic data from available cases was collected and reviewed by expert gastroenterology specialist (JYA). The tumors were then classified as subepithelial tumor like lesion, slightly depressed lesion, and AGC Borrmann types (Table 1) according to the widely accepted endoscopic definition.

### 2.1.3 Histologic assessment and statistical analysis

Hematoxylin and eosin-stained (H&E) sections were available for all cases reviewed, and two pathologists independently evaluated and recorded their gross findings, including histomorphology, pathologic TN stage and lymphovascular invasion status. Any discrepancy was resolved in consensus sessions under a multiheaded microscope. Also, to better characterize gross features, the area of ulcer was grossly measured and divided by the area of the entire tumor in available ulcerated EWDAs. The ulcer proportions of the EWDAs were then compared to those of consecutively resected AGCs from 2010. We used a nonparametric test (Mann-Whitney) to determine the P-value for differences between the groups. Statistical analysis was performed using SPSS software (version 18.0; SPSS, Chicago, IL, USA), with P value $\leq 0.05$ considered statistically significant.

Each case was evaluated for the following histologic features (Figure 1): inharmonious disproportionate glands, irregularly shaped glands, undulating apical mucin border, and markedly distended mucinous cytoplasm. Inharmonious disproportionate glands were defined as glands that are disproportionately larger than surrounding non-neoplastic glands. Irregular glandular shape appeared as structures that did not follow the regular branching contour but was cut off, skewed or distorted. Undulating mucin border was used for irregular, wobbly border of apical mucin caps. Tumor cells with markedly distended mucinous cytoplasm were typically very large and sometimes exceeded 40 times the size of mature lymphocytes.

H&E sections of pretreatment biopsies were available in all cases and were re-evalutated for the presence of EWDA. All specimens were searched for the specific traits described above. The time interval between the initial biopsy and treatment, type of treatment, and the number of procedures performed were recorded. The original diagnoses were also documented and compared with the final diagnoses.

### 2.1.4 Immunohistochemical Analysis

Formalin-fixed paraffin-embedded tissue blocks were available for all 19 cases. To determine the tumor immunophenotype, immunohistochemical staining was performed using antibodies against MUC-2 (1:50, mouse monoclonal, clone Ccp58, catalog No. NCL-MUC-2, Novocastra, Newcastle, UK), MUC-6 (1:200, mouse monoclonal, clone CHL5, catalog No. NCL-MUC-6, Novocastra, Newcastle, UK), CDX-2 (1:500, mouse monoclonal, clone EPR2764Y, catalog No. 235R-16, Cell Marque, Rocklin, California, USA), c-erbB2 (1:8, mouse monoclonal, clone 4B5, catalog No. 790-4493, Ventana, Tusan, USA) and p53 (1:1,000, clone DO-7 Dako), and PTEN (1:100, rabbit monoclonal, clone 138G6, catalog No. 9559, Cell Signaling, Massachusetts, USA). All staining procedures were performed using a Ventana autostainer according to the manufacturer's instructions.

Cytoplasmic staining for mucin core proteins and CDX-2 nuclear staining were considered positive. C-erbB2 staining was evaluated based on traditional HER2 IHC scoring guidelines[3]. Cases scored equivocal for c-erbB2 were tested for HER2 gene copy-number by silver-enhanced in situ hybridization (SISH). For scoring, we followed general guidelines for HER2 copy-number evaluation as described by Jeong et al.[4]. Two pathologists (J.L. and J.K.) independently scored the immunostaining, and any discrepancy was resolved by consensus. Selective next-generation sequencing (NGS) data were available for three cases (Case 2, 3, and 9). NGS was performed according to our routine clinical targeted cancer panel as described previously[5].

## 2.2 Results

### 2.2.1 Clinicopathologic Features

The characteristics of the 19 cases are listed in Table 1. The mean age of the patients was 62.0 years (range, 31 to 81 years) and the male to female ratio was 5.3:1 (16:3). The majority of the tumors (15 of 19, 79.0%) were located in the body of stomach, and the others were located in the cardia (3 of 19, 15.8%) and antrum (1 of 19, 5.3%). Mean tumor size, in the measurable resected cases, was 4.0 cm in the greatest dimension (range, 2.2 to 10.0 cm). Most of the cases (18 of 19, 94.7%) were categorized as AGCs, either clinically or pathologically.

In the 18 cases with available endoscopic data, the majority did not exhibit the typical endoscopic traits of gastric malignancy (13 of 18, 72.2%) (Table 1 and Figure 2). Only one third of them was endoscopically suspected for malignancy showing typical ulcer with infiltration (Borrmann type 3, 3 of 18, 16.7%), ulcer with marginated border (Borrmann type 2, 1 of 18, 5.6%), or diffuse irregular thickening (Borrmann type 4, 2 of 18, 11.1%,). Others were interpreted as slightly elevated lesions (7 of 18, 38.9%), subepithelial tumor like lesions (4 of 18, 22.2%), or a slightly depressed lesion (1 of 18, 5.6%).

Of the AGC cases, the majority were grossly non-ulcerated (11 of 18, 61.1%) (Table 1). They consisted of Borrmann type 4 lesions with an indistinct border and mural thickening (4 of 18, 22.2%), polypoid mass-forming lesions (Borrmann type 1, 4 of 18, 22.2%), and AGC mimicking EGCs (3 of 18, 16.7%). The remaining cases were grossly ulcerated with infiltrative (Borrmann type 3, 6 of 18, 33.3%) or marginated borders (Borrmann type 2, 1 of 18, 5.6%). The EGC (Case 1) was grossly superficially elevated (EGC type IIa). As for the measured gross ulcer proportion, 5 ulcerated EWDA cases and 19 AGCs were available for comparison (Supplement Figure 2, Supplement Table 3). The mean ulcer proportions of the EWDA and AGC groups were

28.0% and 57.3%, respectively. Mann-Whitney test revealed significant differences in the ulcer proportion between the groups (with p-value=0.0048).

Most cases were treated with surgery and adjuvant chemotherapy (11 of 19, 57.9%). Surgery alone was performed in two cases (2 of 19, 10.5%). Four of the 5 initially metastatic cases received palliative chemotherapy (4 of 19, 21.1%). Endoscopic submucosal dissection was performed on the EGC (Case 1, 1 of 19, 5.3%). The majority of the surgically resected AGC cases infiltrated to the subserosa (pT3, 7 of 14, 50.0%). The other cases penetrated to the serosa (pT4a, 4 of 14, 28.6%) or muscularis propria (pT2, 3 of 14, 21.4%). The EGC case invaded to the submucosa (pT1b). Lymphovascular invasion was present in some resected specimens, including the EGC case (5 of 15, 33.3%). Nodal metastasis was histologically identified in some of the surgically resected cases (4 of 15, 26.7%).

Patients were followed up for variable time intervals, ranging from 0.5 to 121 months (mean, 32.0 months) (Table 1). Some patients were alive without evidence of disease at last contact (n=8), while others were lost to follow-up (n=5). Of the 5 patients with distant metastasis at the time of diagnosis, the majority died of the disease at 5 months, 12 months, and 15 months after initial chemotherapy (n=3, Cases 3, 8, and 13). The others were alive with disease at last contact (n=3).

### 2.2.2 Endoscopic evaluation

Eighteen cases which were available for endoscopic assessment were studied for endoscopic impressions (Table1). Only one third was endoscopically compatible with malignancy by expert opinion (JYA) (6 of 18, 33.3%, Borrmann type 3, 3 of 18, 16.6%, Borrmann 4, 2 of 18, 11.1%, Borrmann 2, 1 of 18, 5.6%). The remainder was interpreted as a slightly elevated lesion (6 of 18, 33.3%), a subepithelial tumor (4 of 18, 22.2%), or a slightly depressed lesion (1 of 18, 5.6%). Those cases were not typical for malignancy and looked like neuroendocrine tumor, or gastrointestinal stromal tumor

### 2.2.3 Histologic Assessment

Table 2 shows the histological and immunohistochemical findings of 19 cases. Most of the 19 cases were histologically gastric-type (13 of 19, 68.4%), while the others were intestinal-type EWDAs (6 of 19, 31.6%). Irregular glandular shape was universally present (19 of 19, 100%), and inharmonious glands were present in the majority (11 of 19, 57.9%). About half of the cases showed undulating mucin borders or distended mucins (9 of 19, 47.4%, respectively).

Characteristics worth mentioning in each case were separately recorded (Figure 1). Case 2 exhibited exceptionally large cells with mucin distention approximately 20 times the size of mature lymphocytes. Case 3 showed deceptively benign-looking cells with small nuclei, simulating normal foveolar gland epithelium; it metastasized and the same histology was observed in the patient's omental biopsy. In Case 4, the neoplastic glands were cystically dilated as they progressed toward the serosa. Case 11 exhibited gastritis cystica profunda-like glands infiltrating through the muscularis propria while simultaneously showing large cystic neoplastic glands in the mucosa.

It was also remarkable that in terms of the growth pattern, ulcer formation was relatively small or absent compared to the tumor extent in all 19 cases. Invariably, the mucosal layer was relatively spared, and the epicenter was deeper mucosa to the submucosa. Rather than overt ulceration, the overlying mucosa was involved with frequent glandular cancerization (Figure 1I), or focal mucosal openings leading to large, deeply seated glands (Figure 1D). The latter pattern was reminiscent of lobular endocervical glandular hyperplasia (LEGH) of the uterine cervix. It was also remarkable that desmoplastic reactions were barely observed in all cases.

### 2.2.4 Differentiation from Nonneoplastic Foveolar Glands

Two major features differentiated EWDAs from their benign counterparts, as shown in Figure 3. Irregular spacing of the nuclei was identified as a haphazard location of the

nuclei in terms of nuclear alignment. Careful observation revealed scattered nuclei that were not aligned to the based membrane, that is disrupted polarity. Another feature was disruption of the four lines of the foveolar epithelium, which were formed by the apical mucin cap, base of the mucin cap, cytoplasm and nuclei[6]. Unlike reactive atypia, the tumor cells consistently lacked at least one of the four lines. All 19 cases exhibited these two traits, which were not observed in non-malignant conditions such as hyperplastic polyps or foveolar hyperplasia.

### 2.2.5 Immunophenotype and Association with Histological Features

All 19 cases were variably positive for gastric markers (Figure 4). MUC5AC was faintly (6 of 19, 31.6%) or diffusely expressed (13 of 19, 68.4%). MUC6 was variably expressed in the majority of the neoplasms (11 of 19, 61.1%). Intestinal markers including MUC2 (6 of 19, 31.6%), and CDX-2 (9 of 19, 47.3%), were expressed in some. On an immunohistochemical basis, 13 (68.4%) cases were classified as gastric-type and 6 (31.6%) cases as intestinal-type, which correlated with the histological observations (Table 2). p53 and Ki-67 were overexpressed relative to normal foveolar epithelial cells in all cases (Figure 2E and 2F). C-erbB2 was equivocally expressed in two cases (Cases 2 and 13), but the SISH results were negative in both cases.

There also were a few cases with available targeted cancer panel sequencing results (Cases 2, 3 and 9). Although no consistent feature was found, a few notable mutations were found: NRAS G12D, STK11 Q220Pfs*38 (Case 2), PTEN L108R and Y178C (Case3), and KRAS G12D (Case 9) (Supplement Table 2). Even though the functional significance of the PTEN L108R and Y178C mutations is not known, the loss of PTEN protein expression in this tumor (Figure 4F) suggested that at least one of the two PTEN mutations might be loss of function mutation.

### 2.2.6 Evaluation of Pretreatment Biopsies

One to eight pre-therapeutic endoscopic examinations were performed in 19 patients (median, 2; mean, 2.5). A total of 55 tissue biopsies from 19 patients were available for review. The original diagnoses were ‘adenocarcinoma’ or ‘suspicious for adenocarcinoma’ in 46 biopsies (46 of 55, 84%). Retrospective review of the remaining 11 specimens revealed that 10 of these original biopsies showed histologic evidences for EWDA but the presence of EWDA had been missed at that time. They were diagnosed as benign (6 of 10, 60%), descriptive of atypia (3 of 10, 30%), or favored Menetrier’s disease (1 of 10, 10%) (Supplement Table 1).

## 2.3 Discussion

EWDA consists of bland-looking malignant cells, which causes diagnostic problems in clinical settings and it is not well-described in the literature. In our study, EWDAs were deep-seated tumors with scarce ulceration that were mostly located in the body of the stomach. Four histologic features including irregular glandular shape, undulating apical mucin border, and inharmonious glands and distended mucin were notable features of this neoplasm. Two histological indicators, including irregular nuclear spacing of nuclei and disruption of the four lines, were helpful for recognizing this tricky lesion compared to benign counterparts. We think that our report might help pathologists diagnosing this tricky malignancy.

It was also notable that endoscopic features suggestive of malignancy such as gross ulceration or obvious infiltrating mass[7] were not prominent. Slightly elevated lesions or subepithelial tumor-like lesions were the common impressions of EWDA which are rarely suspected for malignancy [8]. Such endoscopic appearances of EWDA cases might cause further clinical problems because gastroenterologists may not perform immediate repeated biopsy when the initial pathology report does not include “adenocarcinoma” because they are not confident for the presence of malignancy in those settings. In this sense, the timely pathologic diagnosis of this subtle malignancy is

further important.

In contrast to conventional gastric adenocarcinomas, which show ulceration[7], Borrmann type 1 and 4 were more frequently observed in EWDA cases. This is probably because the EWDA grows in an undermining fashion and mucosa is only involved by glandular cancerization. Previous reports have also reported EWDAs as tumors preferentially grow beneath the mucosa to form polypoid masses[1]. LEGH-like glands with distinct lobular arrangements were also notable in our cases. They were situated in the submucosa, which opened up to the mucosa in pinpoints, which was also noted by previous case studies in the literature[1, 2]. These findings might suggest EWDAs originating from a deeper part of the mucosa.

Several histologic features were notable, and irregular glandular shape and undulating apical mucin border were the most consistent findings in our series. Mucin distension resembling mucous neck cells was frequently observed in our cases and have also been described by others [1, 2]. Inharmoniously large glands with marked mucin distension commonly found in our cases were also described by Joo et al. in their EWDA case study [2]. Disruption of the four lines, used to distinguish low grade dysplasia in Barrett esophagus[6], was applicable in our series to distinguish EWDAs from hyperplastic polyps, gastritis cystica profunda, or reactive atypia. We believe that using the four lines and irregular nuclear spacing in combination with the aforementioned histologic characteristics could help in distinguishing EWDAs from mimickers, especially in small biopsy samples.

As for the immunohistochemical findings, MUC5AC and CDX-2 immunolabeling were the most consistent with histological gastric- and intestinal-type EWDAs, respectively. MUC6 and MUC2 expression were not prominent in both subtypes, which was different from the immunohistochemical profile of conventional gastric adenocarcinomas that show diffuse expression of either marker[9]. C-erbB2 expression was not identified in all 19 cases. Ki-67 expression was randomly increased in the carcinoma cells, in contrast to reactive lesions where only basal crypts showed an increased Ki-67 labeling index, consistent with the findings of Niimi et al.[10]. Null- or

diffuse-type mutation pattern p53 immunolabeling found in some tubular adenocarcinomas of the stomach [11] was not identified, but there was an increase against the background gastric foveolar epithelium in all cases, which was also discussed by Niimi et al.[10]. Thus, the combination of p53 and Ki-67 immunohistochemistry might be helpful in biopsy diagnosis provided that they are compared to adjacent nonneoplastic gastric glands.

There were also several limitations to our study. Firstly, the prevalence of EWDA could not be estimated accurately. Initially metastatic cases were missed during surveillance because screened data only consisted of consecutive operated cases and the resulting data could not represent the real prevalence. The referral bias as a tertiary medical institution also contributed to the inaccuracy in estimating the prevalence. Secondly, many patients were lost to follow-up after a few years and prognostic data could not be gathered sufficiently. Proper epidemiologic and clinical behavioral data of this rare neoplasm should be assessed by a prospective study with a larger sample size in the future. Furthermore, NGS study was limited to only a small portion of our cases, and genetic mass data accumulation may show recurring mutations in the future.

# 제 3 장 결 론 (Conclusion)

In conclusion, most EWDAs were grossly non-ulcerative, elevated tumors with an undermining growth pattern. Microscopically, they show mucosal glandular cancerization with a LEGH-like submucosal growth pattern. The histologic features, including disproportionately large, irregularly shaped glands with mucin distention, and undulating apical border were indicative of EWDA. Irregular nuclear spacing and disruption of four lines were helpful features in differentiating gastric type EWDA from reactive foveolar gland hyperplasia. For the timely diagnosis of these deeply seated tumors which are often endoscopically ambiguous, generous forceps biopsies are advised.

# Figure legends

**Figure 1. Representative photomicrographs of EWDA.** (A) Intestinal-type EWDA featuring goblet cells is observed in Case 12. (B) Irregularly shaped glands (arrows) opposed to normal foveolar glands (arrowheads) are observed in Case 4. (C) Inharmoniously large glands (arrows) are noticeable against benign foveolar glands (arrowheads) in Case 4. (D) A focal mucosal opening giving way to to larger, deeply seated glands is noted, resembling LEGH of the uterine cervix (Case 17). (E) Extremely large neoplastic glands at least twenty times the size of normal lymphocytes are noted in Case 2. An undulating apical mucinous border is also observed (arrows). (F&G) Case 3 shows bland-looking gastric type EWDA glands both in gastric (F) and omental biopsy specimens (G). (H) Case 11 shows cystically dilated thin neoplastic glands invading the muscularis propria with gastritis cystica profunda-like portions in the submucosa. (I) Glandular cancerization (arrows) in the background of normal foveolar glands (arrowheads) is more commonly found in EWDA than in conventional gastric carcinomas. (H&E, original magnification, A, 100x, B, 100x, C, 100x, D, 100x, E, 200x, F, 100x, G, 100x, H, 40x, I, 200x).

**Figure 2. Endoscopic appearances of diagnostically difficult EWDA cases**. (A) A mucosal elevation with mostly intact mucosa simulating a subepithelial tumor is noted (Case 1). (B) A slightly elevated lesion with vascular engorgement is noted at angle (Case 6). (C) A slightly depressed lesion is observed in Case 12. (D) Diffusely thickened gastric wall in the gastric body is observed (Case 9). (E) A slightly elevated mass is identified in Case 7. (F) Thickened gastric folds with vascular engorgement (endoscopically Borrmann type 4) are seen in Case 13.

**Figure 3**. **Comparison between EWDA and its mimickers**. (A) Hyperplastic polyp shows an organized gland shape and aligned nuclei. (B) EWDA glands (Case 4) show an irregular glandular shape and irregular nuclear spacing. (C) Normal foveolar epithelium displays regular nuclear spacing with maintenance of the "4 lines" (arrows): line 1—the gastric type mucin vacuole; line 2—the base of the mucin vacuole; line 3—the cytoplasm; and line 4 the nuclei. (D) Case 6 shows large tumor cells with ample mucin, hyperchromatic nuclei, and disrupted 4 lines (H&E, original magnification, A, 200x, B, 100x, C, 400x, D, 400x). (E) P53 expression is markedly increased in the tumor cells in contrast to the background foveolar epithelium of Case 7 (p53 immunohistochemistry, x100, original magnification). (F) Ki-67 in normal foveolar epithelium shows increased expression only along the base of the crypts while the tumor glands (arrows) show a diffuse increase in Case 8 (Ki-67 immunohistochemistry, x100, original magnification).

**Figure 4. Immunohistochemical pattern of EWDA**. (A) Gastric-type EWDA expresses diffuse MUC5AC immunoreactivity in case 5. (MUC5AC immunohistochemistry, x200, original magnification). (B) MUC6 is focally expressed in Case 5 (MUC6 immunohistochemistry, x200, original magnification). (C) CDX-2 shows diffuse nuclear expression in an intestinal type EWDA (Case 7). (CDX-2 immunohistochemistry, x200, original magnification). (D) PTEN is lost in this PTEN mutant large cancer cells in contrast to the normal expression in endothelial cells in Case 3 (PTEN immunohistochemistry, x200, original magnification).

**Supplementary Figure 1.** (A) Inharmonious disproportionate glands(arrows) are shown in the background foveolar epithelium in Case 19 (B) Distended mucin of gastric-type EWDA glands (arrows) is noted in Case 6. (C) Irregularly distorted glandular shape of the EWDA (arrows) is shown with normal foveolar epithelium (arrowheads) in Case 18. (D) EWDA glands demonstrate a wobbly, undulating apical mucin border (arrows) adjacent to the normal foveolar glands (arrowheads) in Case 8 (H&E, original magnification, X200, X200, X100, X200, respectively).

**Supplementary figure 2.** (A) A Borrmann type 3 EWDA (Case10) is found in the

body of the stomach. (B) A case of EWDA is quantified with 2mm grid. Area of ulceration (orange) and area of tumor (blue) are delineated (Case 10). (C) A Borrmann type 3 advanced gastric cancer is the antrum of stomach. (D) Both area of ulceration (orange) and area of tumor (blue) are delineated.

**Tables**

**Table 1. Clinicopathologic features of 19 cases of EWDA.**

| *Case* | *Age (years)* | *Sex* | *Tumor size, greatest dimension (mm)* | *Location* | *Endoscopic impression* | *Mucosal ulceration* | *Macroscopic finding*[a] | *T/N* | *Distant metastasis*[b] | *Lymphovascular invasion* | *Treatment* | *Follow-up (months)* | *Status* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 77 | Female | 33 | body | SET[j] | NI | EGC IIa | T1b/Nx | NI | present | ESD[g] | 17 | NED |
| 2 | 66 | Female | NA | body | SET | NI | Borrmann 1 | T2/N+[d] | present | NA[f] | chemotherapy | 19 | AWD |
| 3 | 31 | Male | NA | body | SET | NI | Borrmann 1 | T3/N+ | present | NA | chemotherapy | 0.5 | DOD |
| 4 | 56 | Female | 50 | body | slight elevation | NI | Borrmann 3 | T3/N1 | NI | NI | surgery[f] | 35 | AWD |
| 5 | 60 | Male | 25 | body | slight elevation | NI | Borrmann 1 | T4a/N0 | NI | present | surgery | 28 | NED |
| 6 | 62 | Male | 40 | cardia | typical Borrmann 3 | present | Borrmann 3 | T3/N3a | NI | NI | surgery | 23 | NED |
| 7 | 39 | Male | 22 | body | slight elevation | NI | AGC mimicking EGC type IIa | T3/N0 | NI | NI | surgery | 26 | NED |
| 8 | 67 | Male | NA | body | Borrmann 4 | NI | Borrmann 4 | T3/N+ | present | NA | chemotherapy | 14 | DOD |
| 9 | 72 | Male | 100 | body | slight elevation | NI | Borrmann 4 | T3/N0 | present | NI | surgery | 26 | AWD |
| 10 | 69 | Male | 40 | body | slight elevation | NI | Borrmann 3 | T3/N0 | NI | present | surgery | 16 | NED |
| 11 | 58 | Male | 33 | body | slight elevation | NI | AGC mimicking EGC type IIa | T4a/N0 | NI | present | surgery | 8 | NED |
| 12 | 61 | Male | 52 | body | slight depression | NI | AGC mimicking EGC type IIc | T4a/N0 | NI | NI | surgery | 15 | NED |
| 13 | 48 | Male | NA | body | Borrmann 4 | NI | Borrmann 4 | T2N+ | present | NA | chemotherapy[h] | 13 | NA |
| 14 | 63 | Male | 57 | antrum | typical Borrmann 2 | present | Borrmann 2 | T3N2 | NI | NI | surgery | 80 | NA |
| 15 | 79 | Male | 33 | body | typical Borrmann 3 | present | Borrmann 3 | T2N0 | NI | NI | surgery[i] | 121 | NA |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 16 | 65 | Male | 65 | body | NA | present | Borrmann 3 | T4aN3 | NI | present | surgery | 70 | NA |
| 17 | 81 | Male | 25 | cardia | SET | present | Borrmann 1 | T3N1 | NI | present | surgery | 79 | DOD |
| 18 | 55 | Male | 33 | cardia | typical Borrmann 3 | present | Borrmann 3 | T2N0 | NI | NI | surgery[i] | 15 | NA |
| 19 | 66 | Male | 30 | body | slight elevation | NI | Borrmann 4 | T2N0 | NI | NI | surgery[i] | 15 | NED |

NED, no evidence of disease; N/A, not available, AWD, alive with disease; DOD, died of disease

[a]Macroscopic finding was classified according to World Health Organization criteria.

[b]At the time of diagnosis

[c]N+, nodal metastasis clinically assessed

[d]NI, not identified.

[e]NA, cannot be assessed

[f]Surgery and adjuvant chemotherapy

[g]ESD, endoscopic submucosal dissection.

[h]Chemotherapy and metastatectomy (right hemicolectomy)

[i]Surgery alone

[j]Subepithelial tumor

**Table 2. Histologic and immunohistochemical features of 19 cases of EWDA.**

| *Case number* | *Histologic type* | *Histologic feaures* | *Immunohistochemistry* | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | *MUC5AC* | *MUC6* | *MUC2* | *CDX-2* | *[a]C-Erb B2* | *[b]p53* |
| 1 | Gastric | S,U | + | mucosa | - | - | - | 1+ |
| 2 | Gastric | M,S,U | + | mucosa | - | - | 2+ | 2+ |
| 3 | Gastric | M,S,U | + | mucosa | - | - | - | 2+ |
| 4 | Gastric | I,M,S,U | + | mucosa | - | - | - | 3+ |
| 5 | Gastric | M,S,U | + | mucosa to submucosa | +, focal | - | - | 1+ |
| 6 | Gastric | M,S | + | mucosa to muscularis propria | - | - | - | 3+ |
| 7 | Intestinal | S | faint+ | - | - | + | - | 3+ |
| 8 | Gastric | M,S,U | + | mucosa | - | - | - | 3+ |
| 9 | Intestinal | I,S | faint+ | - | - | + | - | 2+ |
| 10 | Gastric | I,S | + | mucosa to submucosa | +, focal | + | - | 2+ |
| 11 | Gastric | I,M,S,U | + | mucosa to submucosa | +, focal | +, heterogeneous | | 3+ |
| 12 | Intestinal | I,S | faint+ | - | - | + | - | 3+ |
| 13 | Gastric | I,M,S,U | + | focal | - | faint, focal + | 2+ | 2+ |
| 14 | Gastric | I,S | + | - | +, focal | - | - | 2+ |
| 15 | Intestinal | I,S | faint+ | - | - | + | - | 2+ |
| 16 | Gastric | I,S | + | - | +, focal | - | - | 2+ |
| 17 | Gastric | M,S,U | + | mucosa | +, focal | - | - | 2+ |
| 18 | Intestinal | I,S | faint+ | - | - | + | - | 2+ |
| 19 | Intestinal | I,S | faint+ | - | - | + | - | 2+ |

I: inharmonious disproportionate glands; M: distended mucin; S: irregular glandular shape; U: undulating apical mucin border

[a]C-Erb B2 grading followed the guidelines for HER2 testing issued by the College of American Pathologists

[b]p53 grading, 1+:1-%~1/3; 2+, 1/3~2/3; 3+, >2/3

**Supplement table 1.**

| *Case Number* | *Original Diagnosis* | *Revised Diagnosis* |
|---|---|---|
| 2 | Hyperplastic foveolar epithelium showing dysplasia | Gastric-type EWDA |
| 3 | A few atypical hyperplastic gland clusters, favor reactive | A few atypical hyperplasitc gland clusters, suggestive of adenocarcinoma well differentiated |
| | Markedly hyperplastic atypical gastric foveolar glands with mild nuclear atypia, favor Menetrier's disease | Markedly hyperplastic atypical gastric foveolar glands with mild nuclear atypia, suggestive of adenocarcioma, well differentiated |
| 6 | Atypical glandular proliferation | Gastric-type EWDA |
| 7 | Chronic active gastritis, marked, with intestinal metaplasia, regenerating atypia and scar | Atypical fundic type glandular proliferation with structural atypia, suggestive of adenocarcinoma, well differentiated |
| 11 | Atypically dilated glands in erosive background | Adenocarcinoma, very well differentiated |
| 12 | Atypical metaplastic glands in erosion, favor reactive | Gastric-type EWDA |
| | Dilated benign looking glands in thick disorganized muscularis mucosa, suggestive of gastritis cystica profunda | Gastric-type EWDA |
| 13 | Chronic gastritis, mild with atrophy and foveolar epithelial hyperplasia | Gastric-type EWDA |

**Supplement table 2.**

| *Case number* | *Detected alteration* | *Histologic pattern* | *Stage* |
|---|---|---|---|
| 2 | *NRAS* G12D mutation | Gastric-type | T2N+[a]M+[b] |
| | *STK11* Q220Pfs*38 mutation | | |
| | High *ERBB3* amplification (~ 12 copies) | | |
| | Strong *MDM2* amplification (~ 35 copies) | | |
| 3 | *PTEN* mutations (L108R, Y178C) | Gastric-type | T3N+M+ |
| | *RET* R79Q mutation | | |
| 9 | *KRAS* G12D mutation | Intestinal-type | T3N0Mx |
| | *MYC* amplification (16 copies) | | |

[a]N+, Nodal metastasis, clinically assessed

[b]M+, Distant metastasis, clinically assessed

# Figures

**Figure 1. Representative photomicrographs of EWDA.**

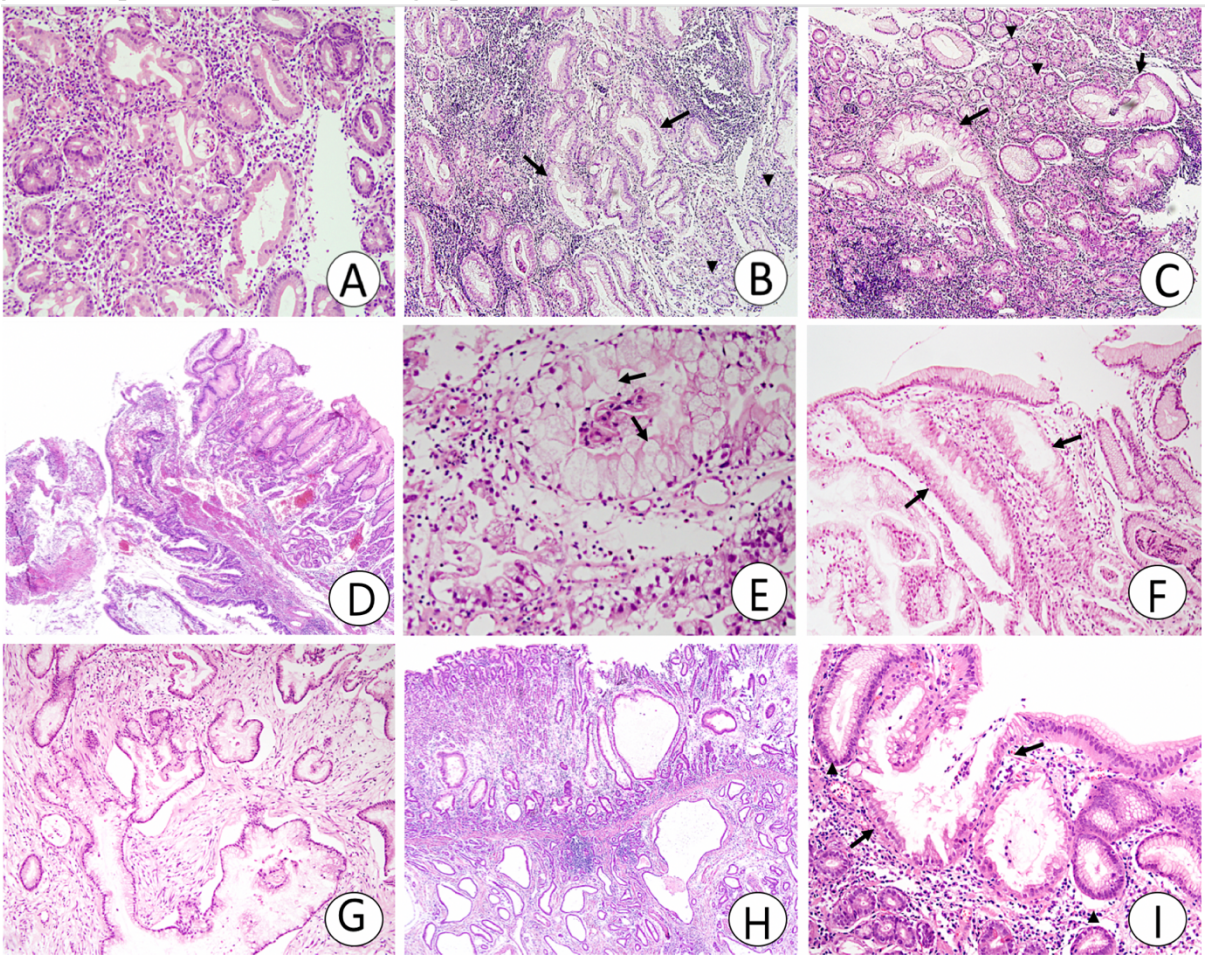


**Figure 2. Endoscopic appearances of diagnostically difficult EWDA cases.**

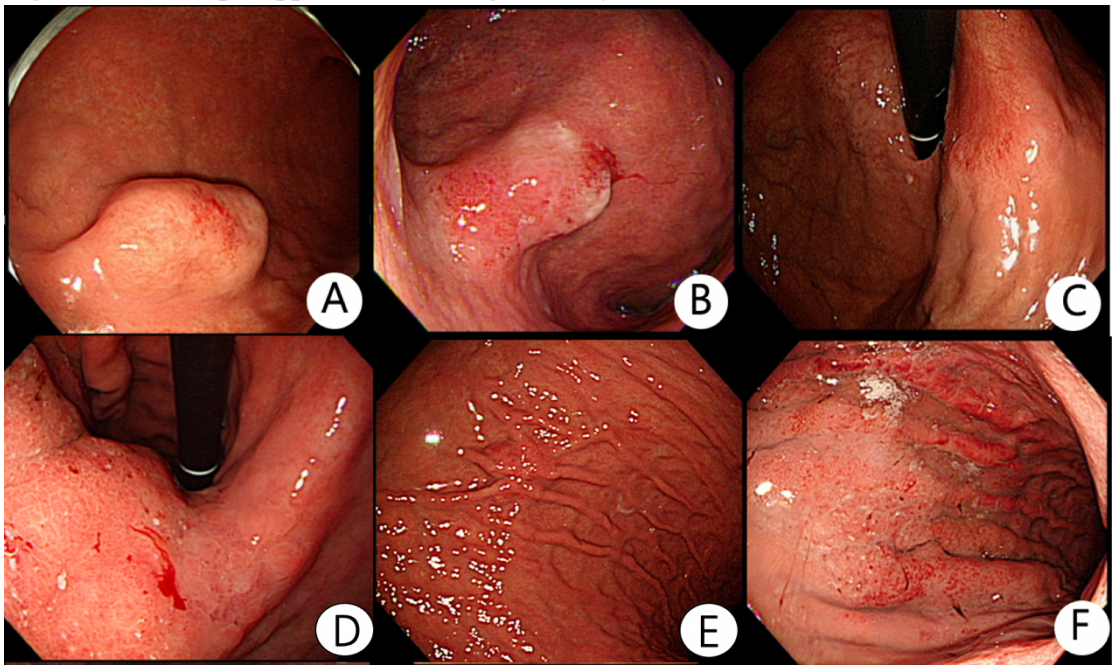



**Figure 3. Comparison between EWDA and its mimickers**

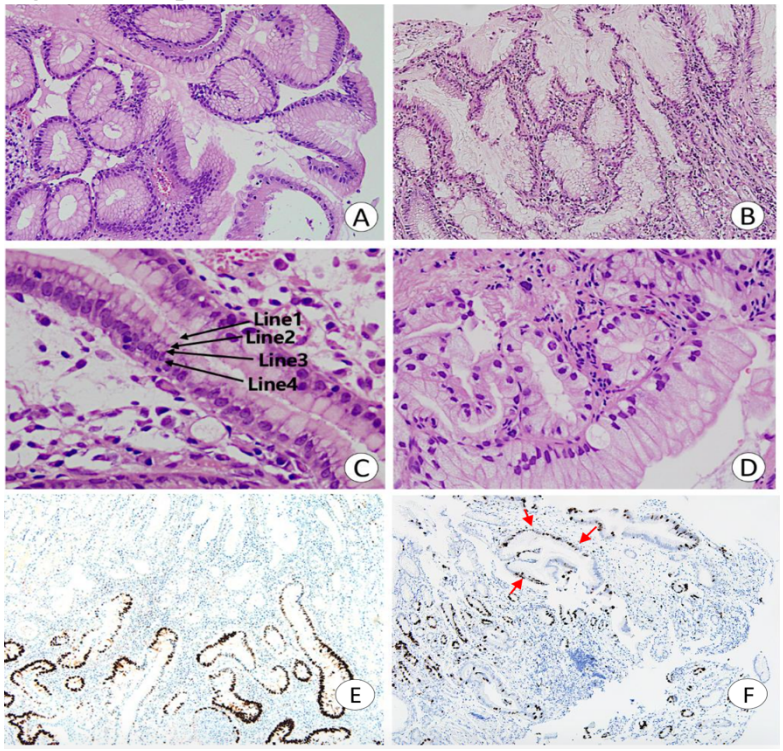
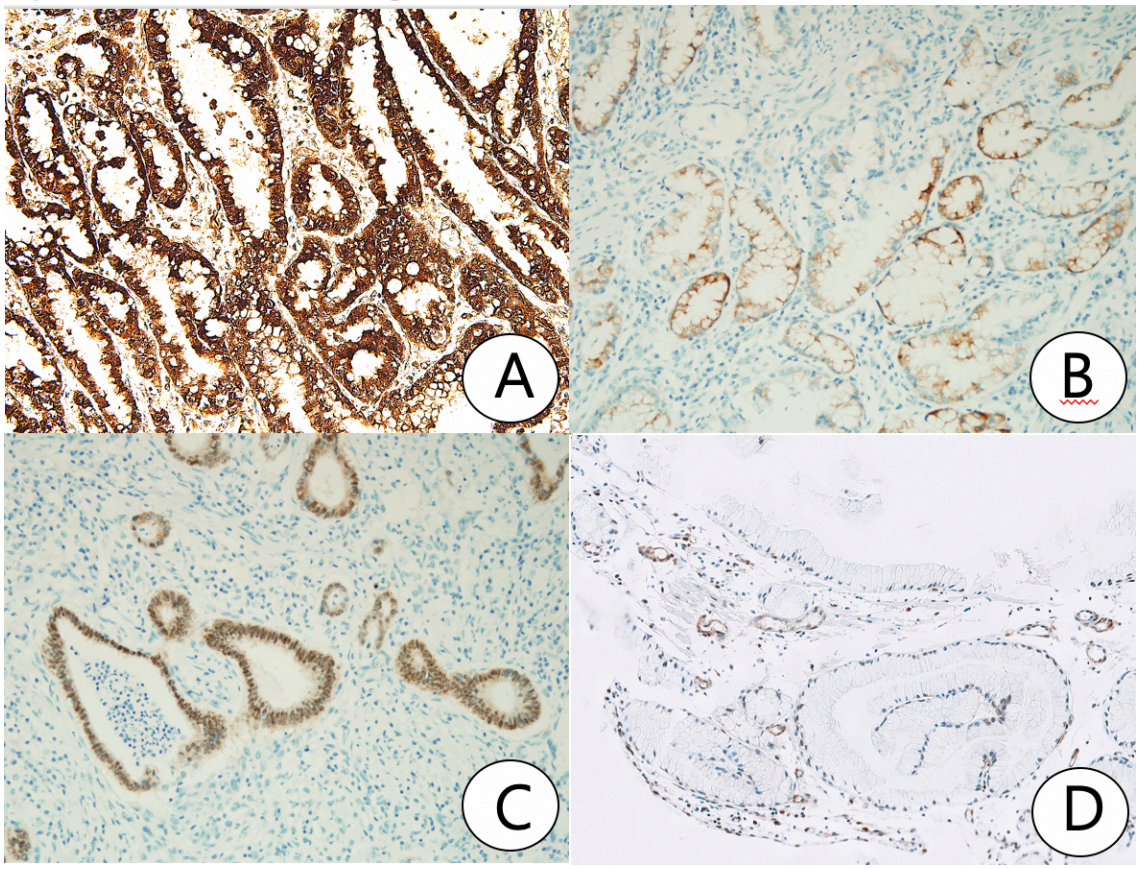
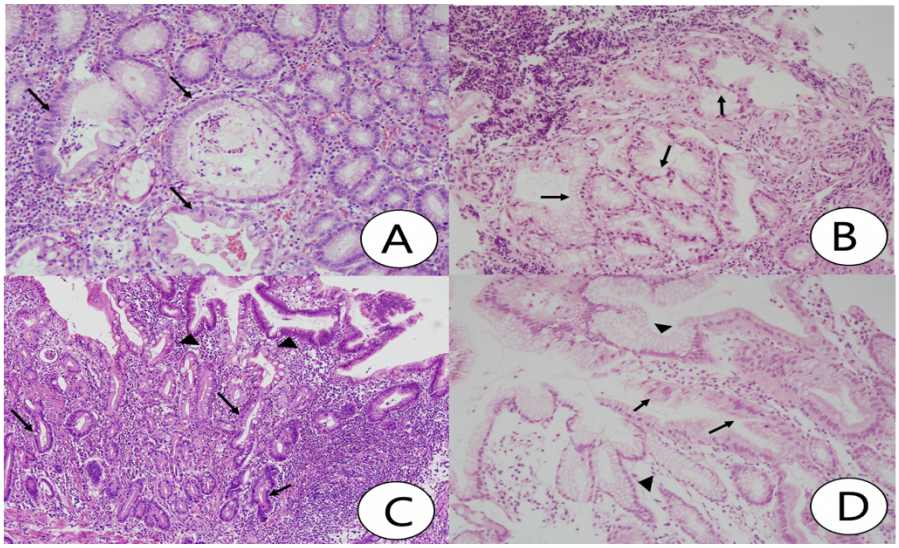


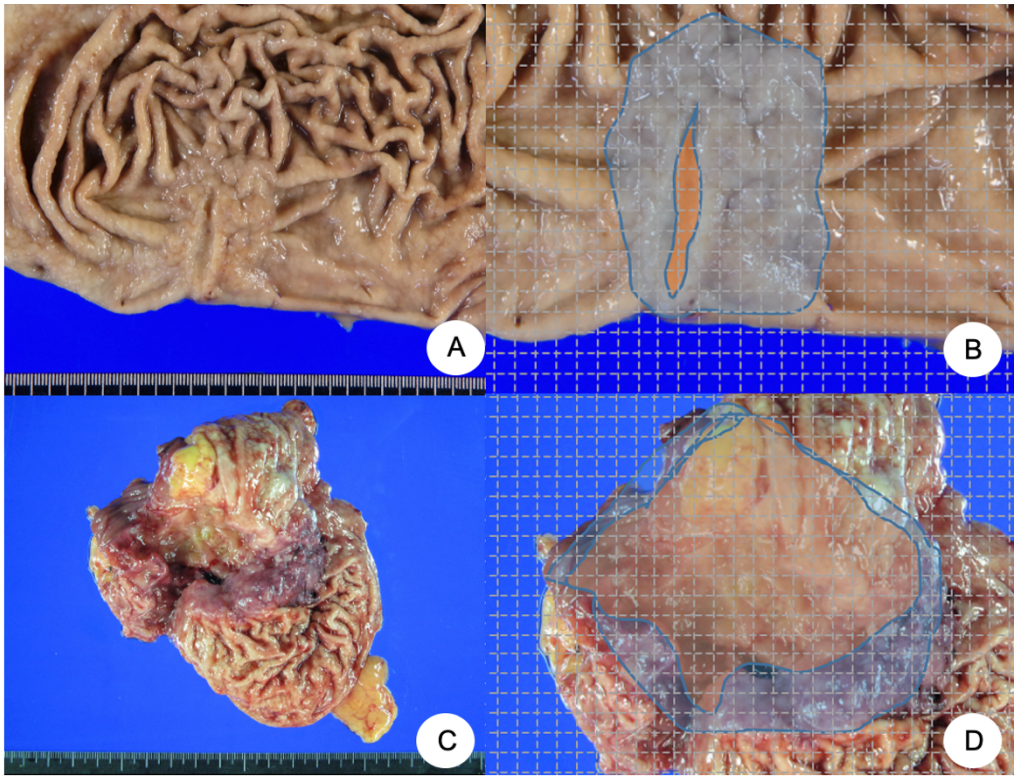
**Figure 4. Immunohistochemical pattern of EWDA.**




**Supplementary Figure 1.**



**Supplementary figure 2.**



# 참고 문헌


1. Yao, T., et al., Extremely well-differentiated adenocarcinoma of the stomach: clinicopathological and immunohistochemical features. World J Gastroenterol, 2006. 12(16): p. 2510-6.
2. Joo, M. and S.H. Han, Gastric-Type Extremely Well-Differentiated Adenocarcinoma of the Stomach: A Challenge for Preoperative Diagnosis. J Pathol Transl Med, 2016. 50(1): p. 71-4.
3. Kim, K.M., et al., Human epidermal growth factor receptor 2 testing in gastric cancer: recommendations of an Asia-Pacific task force. Asia Pac J Clin Oncol, 2014. 10(4): p. 297-307.
4. Jeong, J.H., et al., HER2 Amplification and Cetuximab Efficacy in Patients With Metastatic Colorectal Cancer Harboring Wild-type RAS and BRAF. Clin Colorectal Cancer, 2017. 16(3): p. e147-e152.
5. Hong, Y.S., et al., Src mutation induces acquired lapatinib resistance in ERBB2-amplified human gastroesophageal adenocarcinoma models. PLoS One, 2014. 9(10): p. e109440.
6. Waters, K.M., et al., Refined Criteria for Separating Low-grade Dysplasia and Nondysplastic Barrett Esophagus Reduce Equivocal Diagnoses and Improve Prediction of Patient Outcome: A 10-Year Review. The American Journal of Surgical Pathology, 2018. 42(12): p. 1723-1729.
7. Cree, I.A., c. Centre international de recherche sur le, and s. Organisation mondiale de la, WHO classification of tumours of the digestive system. 2019.
8. Humphris, J.L. and D.B. Jones, Subepithelial mass lesions in the upper gastrointestinal tract. J Gastroenterol Hepatol, 2008. 23(4): p. 556-66.
9. Ushiku, T., et al., Very well-differentiated gastric carcinoma of intestinal type: analysis of diagnostic criteria. Mod Pathol, 2013. 26(12): p. 1620-31.
10. Niimi, C., et al., Usefulness of p53 and Ki-67 Immunohistochemical Analysis

for Preoperative Diagnosis of Extremely Well-Differentiated Gastric Adenocarcinoma. American Journal of Clinical Pathology, 2002. 118(5): p. 683-692.

11. Nakayama, T., et al., Lineage analysis of early and advanced tubular adenocarcinomas of the stomach: continuous or discontinuous? BMC cancer, 2010. 10: p. 311-311.

# 영 문 요 약

**Background**

Extremely we differentiated adenocarcinoma (EWDA) is a deceptively bland-looking adenocarcinoma of the stomach. It often causes diagnostic problems, especially in endoscopic biopsy samples. To better recognize this tricky malignancy, we carefully reviewed a series of EWDAs treated at our institution.

**Design**

A total of 55 specimens from 19 patients were obtained. Gross and microscopic features defining EWDA were described and documented. For comparison, normal surrounding epithelium, regenerative atypia, hyperplastic polyp, and foveolar epithelial hyperplasia specimens were randomly selected and reviewed.

**Results**

Most cases (18 of 19, 94.7%) were advanced gastric cancer (AGC) and primarily located in the body of the stomach (15 of 19, 79.0%). The majority of AGCs were non-ulcerated (11 of 18, 61.1%) with an undermining pattern of invasion and relatively small mucosal involvement. Specific histologic features included an irregular glandular shape, an undulating apical cytoplasmic border, disproportionately large glands, irregularly spaced nuclei and a variably distended mucinous cytoplasm. Classical features, such as small infiltrating glands or desmoplastic reactions, were barely observed. Identification of disproportionately large glands against the surrounding normal glands, indicating glandular cancerization, as well as the atypical cytologic features described above was helpful for making a diagnosis from a biopsy sample. Such subtle signs of malignancy were missed in some of the preprocedural forceps biopsies (7 of 19, 37.0%).

**Conclusion**

Awareness of its histomorphologic characteristics that were described in this report would lead to a more timely diagnosis and would prevent repeated endoscopic procedures.